HUMAN SOLUBLE CD163 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN sCD163 CONCENTRATIONS IN CELL CULTURE SUPERNATES, EDTA PLASMA AND SERUM

THIS PROTOCOL OR DATA IS PROVIDED FOR DEMONSTRATION ONLY. ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY.NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PURCHASE INFORMATION:
THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	HUMAN SOLUBLE CD163 ELISA
Catalog No.	SK00694-01
Lot No.	
Formulation	96 T
Standard range	0.156 - 10 ng/mL
Sensitivity	100 pg/mL
Sample Volume	100 µL
Dilution Factor	200 **(Optimal dilutions should be determined by each laboratory for each application)**
Sample Type	Serum, EDTA plasma, Cell Culture Supernates
Specificity	Human sCD163 only
Calibration	Human sCD163 recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	Refer to lot specific
This kit contains sufficient materials to run approximately 35 samples duplicated provided that assay is run according to protocol.	

Order Contact:
AVISCERA BIOSCIENCE, INC
2348 WALSH AVE., SUITE C
SANTA CLARA, CA 95051
USA
Tel: 408-982-0300
Fax: 408-982-0301
Email: Sales@AvisceraBioscience.com
Info@AvisceraBioscience.com
www.AvisceraBioscience.com

DESCRIPTION

This Human Soluble CD163 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human sCD163 from cell culture supernates, serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human sCD163 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural sCD163 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human sCD163. The capture antibody can bind to the human sCD163 in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human sCD163 is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of human sCD163 bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix reagents with those from other lots or sources.
_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_Each laboratory must determine the optimal dilution factors for the samples being assayed.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
sCD163 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified antibody against human sCD163.	**694-01-01**	**1 plate**
sCD163 Standard – refer to lot specific of recombinant human sCD163 in a buffered protein base with preservative; lyophilized.	**694-01-02**	**1 vial**
Detection Antibody Concentrate – refer to lot specific of concentrate of biotinylated purified antibody against human sCD163 with preservative; lyophilized.	**694-01-03**	**1 vial**
Positive Control – one vial of recombinant human sCD163; lyophilized.	**694-01-04**	**1 vial**
Streptavidin-HRP Conjugate – 120 µL/vial of 100-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 40 mL of buffered protein based solution with preservative.	**DB01**	**1 bottle**
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB68C**	**1 bottle**
Wash Buffer – 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution – 11 mL of 0.5M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 – 8° C for 1 month. For storage up to 10 months, unopened Standard, Positive Control, and Detection Antibody Concentrate should be stored at -20° C or -70° C. For Longer storage for Dilution Buffer (**DB01**), store at -20°C. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20° C or -70° C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C or -70° C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C or -70° C. Avoid repeated freeze-thaw cycles.

Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Serum and plasma samples may require a 200-fold dilution. A suggested 10-fold dilution is 10 µL sample + 90 µL Dilution Buffer. To make a 200-fold dilution, 13 µL of 10-fold diluted sample + 247 µL Dilution Buffer.

Optimal dilutions should be determined by each laboratory for each application.

Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50mL of Wash Buffer Concentrate into deionized or distilled water (450mL) to prepare 500 mL of 1x Wash Buffer.

sCD163 Standard - Reconstitute the sCD163 standard with refer to lot specific of Dilution Buffer. Pipette 250 µL of Dilution Buffer into tubes #1 to #6. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The **10 ng/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/mL).

Tube	Standard	Dilution Buffer	Concentration
stock	powder	Refer to lot	10 ng/ml
# 1	250µl of stock	250µl	5 ng/ml
# 2	250µl of 1	250µl	2.5 ng/ml
# 3	250µl of 2	250µl	1.25 ng/ml
# 4	250µl of 3	250µl	0.625 ng/ml
# 5	250µl of 4	250µl	0.313 ng/ml
# 6	250µl of 5	250µl	0.156 ng/ml

Positive Control - Reconstitute Positive Control with refer to lot specific of Dilution Buffer.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with refer to lot specific of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of **HRP Diluent Solution (DB68C)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution ***(protect from light)***.

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Add 100 µL per well of **Dilution Buffer** to Blank wells.
3. Add 100 µL of **Standard dilutions** in reverse order of serial dilution, **samples,** or **positive control** per

well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.

4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with **1x Wash Buffer** (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µL of **Detection Antibody working solution** to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 1 hour on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of **Substrate Solution** to each well. Incubate for refer to lot on microplate shaker at room temperature. **Protect from light.**
10. Add 100 µL of **Stop Solution** to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Calculation of samples with a concentration exceeding that of standard 10 ng/mL may result in inaccurate, low human soluble CD163 levels. Such samples require further external predilution according to expected human soluble CD163 values with Dilution Buffer in order to precisely quantify the actual human soluble CD163 level.

TYPICAL DATA

This standard curve is provided for demonstration only. A new standard curve should be generated for each set of samples assayed.

Standard (ng/mL)	Average OD450nm (Corrected)
Blank	0 (refer to lot)
0.156	0.029
0.313	0.069
0.625	0.127
1.25	0.249
2.5	0.567
5	1.012
10	1.435

SPECIFICITY

PROTEINS	CROSS-REACTIVITY
Human sCD163	100%
Mouse sCD163	0
Human CD6	0
Human sTweak	0

LINEARITY

To assess the linearity of the assay, pooled research human **EDTA plasma** samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
200X	1.9897	397.94	100
400X	1.0057	402.28	101.1

To assess the linearity of the assay, pooled research human **serum** samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
200X	2.2475	449.50	100
400X	1.0863	434.52	96.7

CITATIONS:

Swaminathan A, et al. Plasma Interleukin-27 (IL-27) Levels Are Not Modulated in Patients with Chronic HIV-1 Infection. PLoS One. 2014; 9(6): e98989. Published online 2014 Jun 4. doi: 10.1371/journal.pone.0098989

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or positive control to the well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate 1 hour on plate shaker at RT. **Protect from light**.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate refer to lot specific on the plate shaker at RT. **Protect from light**.
↓
Add 100 µl Stop Solution to each well. Read at 450nm.